Hantavirus Seroconversion of Wild-Caught *Peromyscus* During Quarantine

To the Editor: In 1993 an outbreak of unexplained respiratory deaths in the Four Corners region of the United States led to the discovery of Sin Nombre (SN) hantavirus and the associated human disease, hantavirus cardiopulmonary syndrome (HCPS). Numerous studies have shown that a series of hantaviruses similar to SN virus are maintained in natural reservoirs composed of Sigmodontine rodents, including deer mice (*Peromyscus maniculatus*), white-footed mice (*P. leucopus*), cotton rats (*Sigmodon hispidus*) and western harvest mice (*Reithrodontomys megalotis*) (1). Deer mice, however, are the principal reservoir of SN virus, the primary etiologic agent of HCPS in North America.

Some hantaviruses, thus far not including SN virus, have been transmitted in indoor animal-care facilities through the airborne route (2). The high case-fatality ratio of HCPS (40%), coupled with its airborne transmission by captive rodents, has led to classification of the agents of HCPS as biosafety level 3 (BSL-3) in tissue culture and BSL-4 in reservoir host rodents. Although deer mice mount an antibody response and develop chronic infection, the virus does not harm them. Deer mice are believed to shed SN virus in urine, feces, and saliva. Infection in humans occurs primarily by inhalation of aerosols from dried excreta containing infectious virus, particularly in closed spaces with poor ventilation (3).

Handling mice infected with SN virus in a laboratory requires BSL-4 conditions (4). However, outdoor standards greatly reduce costs and difficulties associated with handling infected rodents safely, since workers wearing respirators and protective clothing may handle infected mice outdoors (5). Thus, we have constructed outdoor quarantine facilities for the temporary housing of potentially infected mice (6). These facilities consist of a series of individual nest boxes enclosed by a partially buried steel plate fence. Mice are placed into individual nest boxes spaced 3 m apart, which prevents transmission of hantavirus among mice during quarantine (J. Botten and B. Hjelle, unpub. data). Each nest box is composed of an artificial burrow enclosed within a small steel container, which serves as a barrier to contain each mouse. These facilities allow safe handling of wild rodents at much lower cost than that associated with BSL-4 laboratories. Very few, if any, patients with HCPS contracted the virus in an open, outdoor environment (3).

Viral infections are characterized by a window period during which the host is infected but diagnostic test (e.g., antibody) results are negative. To detect infections reliably, it is important to conduct antibody tests after the host animal has been given sufficient time to mount a detectable immune response. Mills et al. (5) recommend testing captured rodents for hantavirus antibodies at the beginning and end of a 5-week quarantine period whenever potential reservoir species are used to establish laboratory colonies. Only upon completion of the second test can an animal be considered truly uninfected by a hantavirus.

We describe two cases of seroconversion in *Peromyscus* spp. that were undergoing such quarantine. These results support the use of a quarantine period in combination with hantavirus antibody testing to clear mice for indoor use.

We collected 132 white-footed mice from one southern and two northern areas of Illinois that have not previously been examined for the presence of hantavirus. The average seroprevalence among these populations was 1.5%. Forty-six of these mice were quarantined for 5 weeks (6), and one mouse underwent seroconversion as detected by strip immunoblot assay. The presence of viral RNA in this mouse was confirmed by reverse transcriptase-polymerase chain reaction (RT-PCR) from lung tissue. In addition, we collected 69 deer mice from an area of New Mexico that had an overall seroprevalence of approximately 20% and placed them in quarantine (6).

One deer mouse delivered four pups while in quarantine and seroconverted 19 days after delivery (6,7). While all four pups were seropositive, viral RNA was detected in the dam by using RT-PCR for lung tissue and immunohistochemistry for heart, lung, and liver tissue (data not shown). Infectiousness of the virus from this mouse was demonstrated by successful passage through uninfected deer mice (7). The fact that the New Mexico pups had not become infected when they were euthanized at 21 days supports other epidemiologic data that suggest that deer mice do not transmit the virus vertically (9-11). These results strongly support the recommendations promulgated by Mills et al. (5) and the Centers for Disease Control and Prevention that wild rodents be used as colony founders only if they remain seronegative for hantavirus after a 5-week quarantine period.

Working in outdoor quarantine facilities is labor-intensive and requires routine maintenance and occasional repair. Building costs depend on the number of nest boxes, but the material cost of a substantial quarantine facility is $10,000 to $20,000. However, safety concerns and the difficulties of maintaining mice alive outdoors without bringing them indoors necessitate their use. A possible exception could be made for very temperate climates, where outdoor cages might be used temporarily.

Our finding that even a recently infected dam, one known to be infectious by horizontal route, did not transmit virus to her pups supports lack of vertical transmission of SN virus as argued previously by workers using less direct methods (9-11).

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References

1. Schmaljohn C, Hjelle B. Hantaviruses: a global disease problem. Emerg Infect Dis 1997;3:95-104.
2. Tsai T. Hemorrhagic fever with renal syndrome: mode of transmission to humans. Lab Anim Sci 1987;37:428-30.
3. Hjelle B, Glass GE. Outbreak of hantavirus infection in the Four Corners region of the United States in the wake of the 1997-98 El Niño-Southern oscillation. J Infect Dis 2000;181:1569-73.
4. Centers for Disease Control and Prevention. Laboratory management of agents associated with hantavirus pulmonary syndrome: interim biosafety guidelines. MMWR Morb Mortal Wkly Rep 1994;43(RR-7):1-7.
5. Mills JN, Yates TL, Childs JE, Parmenter RR, Ksiazek TG, Rollin PE, et al. Guidelines for working with rodents potentially infected with hantavirus. Journal of Mammalogy 1995;76:716-22.
6. Botten J, Nofchissey R, Kirkendoll-Ahern H, Rodriguez-Moran P, Wortman IA, Goade D, et al. Outdoor facility for quarantine of wild rodents infected with hantavirus. Journal of Mammalogy 2000;81:250-9.
7. Botten J, Mirowsky K, Kusewitt D, Bharadwaj M, Yee J, Ricci R, et al. Experimental infection model for Sin Nombre hantavirus in the deer mouse (*Peromyscus maniculatus*). Proc Natl Acad Sci U S A 2000;97:10578-83.
8. Green W, Feddersen R, Yousef O, Behr M, Smith K, Nestler J, et al. Tissue distribution of hantavirus antigen in naturally infected humans and deer mice. J Infect Dis 1998;177:1696-700.
9. Mills JN, Ksiazek TG, Ellis BA, Rollin PE, Nichol ST, Yates TL, et al. Patterns of association with host and habitat: antibody reactive with Sin Nombre virus in small mammals in the major biotic communities of the southwestern United States. Am J Trop Med Hyg 1997;56:273-84.
10. Bennett SG, Webb JP Jr, Madon MB, Childs JE, Ksiazek TG, Torrez-Martinez, et al. Hantavirus (Bunyaviridae) infections in rodents from Orange and San Diego counties, California. Am J Trop Med Hyg 1999;60:75-84.
11. Borucki MK, Boone JD, Rowe JE, Bohlman MC, Kuhn EA, DeBaca R, et al. Role of maternal antibody in natural infection of *Peromyscus maniculatus* with Sin Nombre virus. J Virol 2000;74:2426-29.